A STUDY OF
4 S NEUROBLASTOMAS

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CERTIFICATE

Certified that the dissertation entitled "**A Study of 4 S Neuroblastomas"** , is the original work undertaken by **Dr.P.Elanthirayan**, under our guidance and supervision in the Department of Paediatric Surgery ,Institute of Child Health and Hospital for Children, Madras Medical College & Research Institute, Chennai-3.,during the period of his Post graduation in M.Ch Paediatric Surgery from 2003 to 2006.

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INTRODUCTION

Neuroblastoma is one of the commonest solid tumor in the paediatric age group with an overall poor prognosis. Stage 4S Neuroblastomas as the S stands for is a special group among neuroblastomas. Interesting to note that age is a major criteria which differentiates between two groups of patients at either end of the prognostic spectrum.The neuroblastomas presenting after 1yr with all other features of 4 S seem to behave so,different from the actual 4S Neuroblastomas. These 4S NBs have many unique characteristics. Most important of them being tumor regression and tumor maturation.

Another interesting fact about these tumors is that it defies the conventional definition of metastatic disease.Because in 4 S NB's despite their widespread metastasis prognosis is considered as good and the chances that such tumors will regress is very good.

There have also been disputing claims that these are incidentalomas and cannot be classified as tumors However there are enough studies to prove that these are true tumors very similar

to their non 4 S counterparts and are capable of metastazing and even sometimes progressing rapidly ,with poor outcome.

Though most of these tumors regress and disappear some progress on to stage 4 with resultant poor prognosis.There are few pointers to predict tumor progression and regression.This phenomenon is much less studied and understood.This study aims to evaluate the natural course of the disease and to identify the changing patterns of disease regression and maturation.

The present scenario of unpredictable disease pattern and behavior gives rise to the question of whether to treat these individuals or not. Chemotherapy with its attendant complications in this age group is very risky.On the other hand, to wait and watch a tumor which has metastasized is a difficult decision.There have been various studies supporting and opposing chemotherapy in 4 S Neuroblastomas in the literature.This study aims to formulate a protocol for identifying between the cases to be treated from those which need to be just observed and followed up.In our setup,where molecular studies and genetic markers are not easily available,we aim to formulate a simplified selection criteria,based on the available resources to identify the patients requiring chemotherapy.

REVIEW OF LITERATURE

Neuroblastomas is the most common malignant tumor of neonates and the second most common solid tumor in children.The prevalence is 1 per 7000 live births,overall with slightly lesser prevalence in whites.

It has a slight male preponderance with a male to female ratio of 1:2:1The median age at diagnosis is around 22months. 36% of the patients are less than 1 year at diagnosis. Studies show a biphasic age incidence with an initial peak before 1 year and a second peak between 2 and 4 years.

Environmental Influence

Studies show an association between maternal exposure to drugs like diuretics and neurally active drugs, like Phenobarbital and phenytoin.Two other studies have shown association between paternal exposure to electromagnetic fields and neuroblastomas.These studies however have not been confirmed.

Genetic predisposition:

A subset of patients with neuroblastoma exhibit an Autosomal dominant form of inheritance. Knudson and Strong postulate that 22% of all neuroblastomas are due to a germinal mutation.According to Knudsons hypothesistwo serial mutations occurring in a single cell induces malignant transformation in the cell. Hereditary tumors occur in patients in whom the first mutation is a prezygotic (germinal) event. Only one additional mutation is needed in these persons to induce malignant transformation. Such persons have a higher incidence of neuroblastoma and a peak incidence at an early age. In addition,they may develop tumors at multiple sites and bilateral tumors.

The median age at diagnosis of familial neuroblastomas is 9 months in contrast to the 22 months in general population.If such persons survive, half of their offsprings should be carriers of the geminal mutation with atleast a 63 % chance of developing neuroblastoma.The concordance for neurobastoma in monozygotic siblings during infancy suggests that hereditary factors may be predominant ,whereas the discordance in older twins suggests that random mutations or other factors may play a role.

Chromosomal Abnormalities:

Reports describe constitutional abnormalities involving the short arm of chromosome 1 ,which is frequently deleted or rearranged in neuroblastoma cells.The break point is at 1p 36.This break point may interrupt a gene on 1p 36that is important in malignant transformation.

Associated Genetic Syndromes:

Neuroblastoma is associated with neurofibromatosis type I and aganglionosis of the colon. All these are neurocristinopathies which may represent a maldevelopment of the neural crest.

Cellular and Molecular Pathogenesis :

Studies at cellular and Molecular level in neuroblastoma have led to better means of diagnostic subclassification. Besides,they also provide better means of understanding and predicting the clinical behavior and following up the disease activity in these patients. In addition to providing insights into mechanisms of malignant transformation and progression ,these studies promise to generate novel approaches to treatment.

Embryology :

Studies show crests of neuroblastoma cells occur within the fetal adrenal that may be identified in neonates and infants.These nodules occur as part of normal Adrenal development, peak between 17 and 20 weeks of gestation and gradually regress by birth or shortly thereafter.

Thus , the neuroblastic nodules identified in 3 month old infants who died of other causes were likely remanants of fetal adrenal development.

However,it has been proven beyond doubt that cells in these crest are the ones from which adrenal neuroblastomas develop.The factors that induce the changes which predispose to development of Neuroblastoma from these cells are not completely understood.

These microscopic nodules however are unlikely to be detected clinically or by screening for the urine VMA ,as done in mass screening programmes.

The concept of “in situ “ neuroblastomas and the “spontaneous regression“ of 4 S Neuroblastomas have been

variously argued to represent an extension of this normal phenomenon of adrenal crest development and regression. Though the ‘ in situ ‘ neuroblastoma can be explained so ,the 4 SNB do not fit into this category and represent an exclusive group themselves.

Neuroblastoma cells are derived from postganglionic sympathetic neuroblasts. They sometimes exhibit features of neuronal differentiation. Indeed,neuroblastomas may show spontaneous or induced differentiation to ganglioneuroblastoma or ganglioneuroma. Thus malignant transformation of these cells may result in part from failure to respond fully to normal signals to undergo morphologic differentiation.

Markers of neuronal differentiation have been identified which when present indicate that the tumors are likely to be more differentiated .The nerve growth factor (NGF) is a member of a family of neurotropins which include brain derived neurotroptic factors, neurotropin 3,4,5. Tyrosine kinase receptor genes (Trk A,B,C) have been cloned which encode for the receptors for these neurotropins. Of these, Trk A gene is most commonly observed in neuroblastoma. Presence of Trk A gene is associated with tumors which are likely to differentiate and thus represent a favourable prognosis.

Two other markers of neuronal differentiation have been identified –Chromogranin A and Neuropeptide Y.apart from these somatostatin and VIP are peptide hormones which are associated with good prognosis in neuroblastomas and might serve as indirect evidence of differentiated tumors.

Tumor Karyotyping :

Tumor cells may show 1 p36 deleton on karyotyping .This however carries no importance as far as the disease progression or treatment plan is concerned.

DNA Index:

Flow cytometric analysis of DNA content gives useful information on prognosis and response to chemotherapy .Diploid tumors have a DI of 1 and are associated with poor prognosis .Hyperdiploid tumors with a DI of > 1 are likely to have a better prognosis especially in the infancy age group.

N-myc Amplification:

Gene amplification of specific areas especially the short arm of chromosome 2 which contains the N-myc protooncogene is associated with advanced disease ,whereas the reverse is true for stage 1,2 and 4S disease. Also N-myc is present at time of presentation and does not change with the progresson of disease.

1p36 deletion:

This is found in 70 to 80% of Diploid tumors. Molecular studies indicate a strong correlation between 1p36 deletion and N-myc Amplification.

Pathology of Neuroblastomas

It is one of the “small blue round cell “,neoplasms which includes Ewings,NHL,PNET and RMS. Neuroblastomas arise from primitive pluripotent sympathetic cells which are derived from the neural crest.The pleuripotent sympathogonia which are derived from the neural crest migrate and undergo varing degree and type of differentiation to generate different normal tissues of the sympathetic nervous system including the Adrenal chromoffin cells and the spinal sympathetic ganglia.

The histological subtypes of neuroblastic tumors appear to correlate with the normal differentiation patterns of the sympathetic nervous system.

The three recognized classical patterns of Neuroblastoma, Ganglioneuroblastoma and Ganglioneuroma reflect a spectrum of maturation and differentiation.

The neuroblastoma is composed of small, uniform cells containing dense hyperchromatic nuclei and scant ctytoplasm.The presence of neuritic processes or neurophil is pathognomonic. The Holmer Wright pseudorosette is another diagnostic feature of neuroblastoma seen in 15 to 50 % cases.It is composed of neuroblasts surrounding the eosinophilic neurophil.

At the other end of the spectrum the ganglioneuroma is composed of mainly ganglion cells,neurophil and schwann cells. Ganglioneuroblastomas are a heterogenous group with histopathological features spanning between the two.Ganglioneuroblastoma may be either focal or diffuse with the diffuse ones being less aggressive.

Once a diagnosis of small round cell tumor is made,there may still be some doubt in confirming the diagnosis when the classical pseudorosette or neurophil are not identified.

At this point immunohistochemsitry and electron microscopy help. Neuroblatoma is positive for neurofilaments synaptophysin and neuronspecific enolase,by immunohstochemistry.Electron microscopy typically demonstrates dense core membrane bound

neurosecretory granules as well as microfilaments and microtibules within the neurophil.

Definition of 4 S Neuroblastoma.:

It is defined as stage I or II disease with spread limited to the liver,skin, bone marrow (less than 10 % involvement), in a child under 12 months of age.Bone cortex is not involved. The ‘’S” in 4S neuroblastomas represents ”special “These represent a unique subgroup within the neuroblastomas which show spontaneous regression without any treatment.

Diagnostic Criteria :

1.Stage I or II disease proven by histopathology ,ie, Tissue from primary or Bone marrow ± Urine VMA studies.

2. Absence of disease elsewhere apart from liver ,skin and bone marrow.This has to be confirmed by an USG / CT Scan abdomen /Xray chest / skeletal survey.

3. Age < 1 year at presentation.

In only 1-2% of the patients does the disease progress to stage

The major risk for infants with stage 4 S disease comes from systemic effects of the disease ,chief among them being respiratory distress caused by a massively enlarged liver.Other risk factors being Coagulopathy ,renal failure,vomiting and all caused by the enlarged liver. The risk of such systemic effects is much

higher in infants <3 months of age. It is for this reason they are considered as a high risk group within the 4 S NB group.

The prognosis of 4 S NB is good with an overall survival of 85 to 90%. DeBernandi found out that cases presenting < 6 months of age did better than those between 6 and 12 months of age (92 % Vs 80 %).

The commonest mode of presentation is an abdominal mass. Adrenal is the MC site of primary 60(%).In 5..% cases, however a primary could not be detected .Among the three secondary sites (Liver,Skin & BM),presence of subcutaneous nodules indicates a good prognosis .Urine VMA is detectable in 90 to 95 % of cases.

Prognostic Factors :

The prognostic factors for 4 S NB is same as that for all neurobblastomas. Of all the prognostic factors some carry a greater significance.

The Shimada histopathological grading is one based on the patients age along with three histopathological factors.

1. Presence or absence of Stroma.
2. Degree of differentiation.

3. Mitosis –Karyorrhexis index.

Generally age < 1 year ,stroma rich well differentiated tumors,MK Index of <100 indicate good prognosis.

Joshi et al have proposed three grades based on two factors namely presence of calcification and mitotic rate.Less than 10 mitoses / 10 HPF (indicates low mitotic rate)

Grade I : Calcification present / Low mitotic rate.

Grade II : either one only present.

Grade III : Both absent.

Apart from these histological criteria genetic markers like N-myc amplification,1p36 deleton and DNA index are of great prognostic significance.

N-myc is a protooncogene situated in the short arm of chromosome 2.This genes when amplified are identified as homogeneously straining region within the chromosme. Nmyc amplification is associated with predominantly advanced stage,rapidly progressing disease and other poor prognostic factors.

Amplification is found in 5 to 10 % of allpatients with 4 S .But m, it is noted in 30 to 40 % of those in the 4 S group which progress to stage 4. N-myc is present from the time of diagnosis and does not change with the course of the disease.Thus it serves as a good predictor of disease behaviour.

Flow cytometry analysis of measures total cell DNA content.Diploid tumors have a DI of 1.Hyperdilpoid tumors with DI . >1 are likely to have low stage of disease and respond better to cyclophosphamide and doxorubicin.Diploid tumors (DI = 1) on the other hand are poor responders.

Loss of Heterozygosity for 1 p is another factor associated with poor prognosis.

Children's oncology group ,North America has classified the neuroblastoma cases based on tumor biology.The three criteria being considered are N-myc Amplification, shimada histology and DNA ploidy.

Low risk 4 S Neuroblastoma.

N-myc not amplified.

Favourable shimada histology.

Hyperdiploid DNA.

Intermediate risk 4 S Neuroblastoma.

N-myc not amplified.

Unfavourable shimada histology

And / or

Diploid DNA

High risk 4 S Neuroblasstoma :

N-myc amplified.

Apart from these few other variables also serve as markers of prognosis.

- Age >6 months at presentation, likelihood of progressing into stage 4 disease.
- Elevated Serum ferritin is associated with poor prognosis because they may indicate an increased tumor load.On the other hand ferritin may be needed for growth of NB cells.
- Neuron specific enolase activity is directly proportional to the neural cells.Elevated levels indicate rapidly progressing Active tumor.However NSE is not specific for neuroblastomas and may be elevated in other tumors also.

- GD2 is the most characteristic ganglioside on neuroblastoma cells.It can be also detected in the serum and useful as a marker of disease activity and response to treatment.
- Increased Serum LDH levels may reflect rapid cellular turnover and is a non specific marker.
- Recently NDGF Receptor expression and TRK –A gene expression have been proven to be associated with maturation of Neuroblastoma cells.They,thus play a role in disease regression in 4 S NB and indicate a good prognosis.

Mass Screening for 4 S Neuroblastomas

This was introduced in Japan 20years ago, to identify tumors at early stage and to treat them aggressively . They consists of mass screening of all infants at 6 months of age for Urine VMA levels through kits supplied to parents.This is then supplemented with Urine HVA / DA at 1 ½ years of age in certain studies to pick up cases missed in the initial screening .This screening programme increased the incidence of neuroblastomas detected many fold in this age group. However long term studies have shown that more than half of these tumors detected by

screening regress spontaneously .Thus the very purpose of early detection and aggressive treatment is affected.

Studies have shown that tumors presenting at < 6 months of age are of favourable prognosis.Same holds good for cases detected by screening. Moreover it is the biological characteristics of the tumor which determine the final outcome. Thus even if tumors are detected by screening the biological prognostic factors should be studied.

Aggressive treatment should be started only for those in the high risk category.

Disease pattern :

Excepting for the 1 to 2 % of cases which progress on to stage 4 rest of the 4S Neuroblastomas regress spontaneously . This process of regresson however has been poorly understood.No factors causing regression have been identified till now.However it has been proven beyond doubt that regression occurs by **Apoptosis**,ie, programmed cell death.But what causes it and how is still under evaluation.

The other phenomenon of tumor maturation which is a process of differentiation of neuroblastoma cells is better understood.The presence of nerve derived growth factors in the presence of their receptors induces the cells to undergo differentiation.Thus the cells start differentiating first to the ganglioneuroblastoma and then on to the benign ganglioneuroma form.The NDGF receptors being carried by the Trk A genes. Tumor biology for these genes gives a fair idea of predicting which of these tumors will mature.

Management Strategies:

There has been considerable controversy over whether to treat 4 S neuroblastomas actively or not. Studies supporting as well as opposing active intervention have been published .the rationale for chemotherapy in these patients is to prevent the disease from progressing onto stage 4 and to induce tumor regression.This however is countered by the fact ,the main causes for morbidity and mortality in this group are the secondary effects of the tumor and chemotherapy related complications rather than the disease itself.

Thus the only definitive indication for chemotherapy in these cases is life threatening complications like respiratory distress,coagulopathy etc.However based on molecular studies ,the disease has been categorized into three groups for favour of devising treatment protocol.

Based on the North American cancer study group the treatment protocol is as follows.

- **Low risk** :

  Chemotherapy only for life or organ threatening complications that cannot be relieved by surgical excision.

- **Intermediate risk :**

  24 weeks of chemotherapy,consisting of moderate doses of cyclophosphamide , carboplatin, doxorubicin, etoposide. Radiotherapy to be given if residual disease present after chemotherapy.

- **High risk**

  24 weeks of high doses of the same drugs with inclusion of ifosphamide and cysplatin.For residual disease surgical excision and / or radiotherapy followed by myeloablative CT /RT and stem cell transplant.

Future Consideratons :

- To identify persons with a genetic predisposition to develop this disease.1p36 is one locus which has been studied.Further studies to confirm the presence of other loci and to identify the predisposing loci are on and should be of great value in the future.

- The present mass screening programme at 6 months has not proven to be very effective as it detects > 50 % of masses likely to regress.Thus a newly devised screening programme done at later age (10 to 18 months) using other catecholamines as well will improve the efficiency of such screening.

- Urine VMA ,serum ferritin etc as tumor markers are not as reliable and as specific as alpha FP and beta HCG for other tumors.The development of molecular markers like N myc Amplification,1 p deletion,TRK –A expression,however are highly specific but not easily available.Thus further studies to identify specific markers of signficant prognostic value are needed .

- Further studies into the mechanism of tumor regression and maturation might provide insights into the pathology and behaviour of the disease .This might in turn help in devising specific treatment strategies with biologically directed therapy to replace the current toxic regimens.

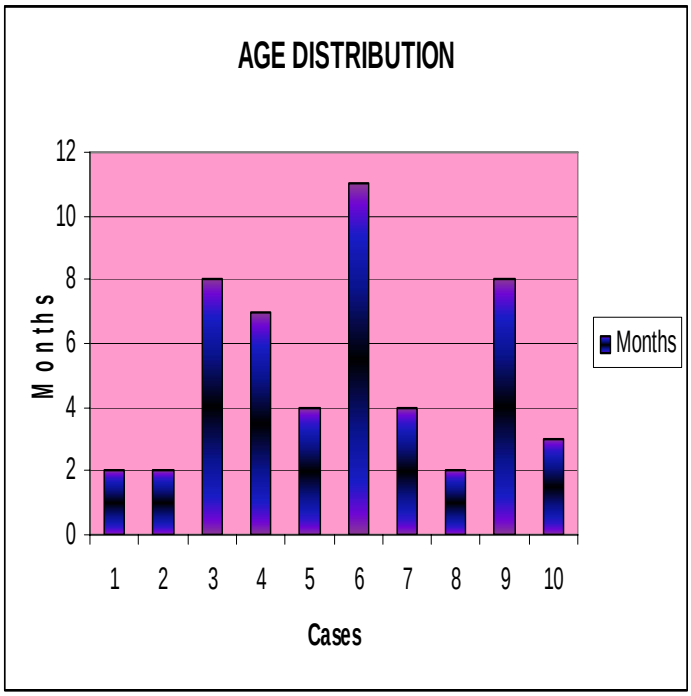
AIM

- To study the natural course of disease and its regression pattern in 4 S Neuroblastomas.

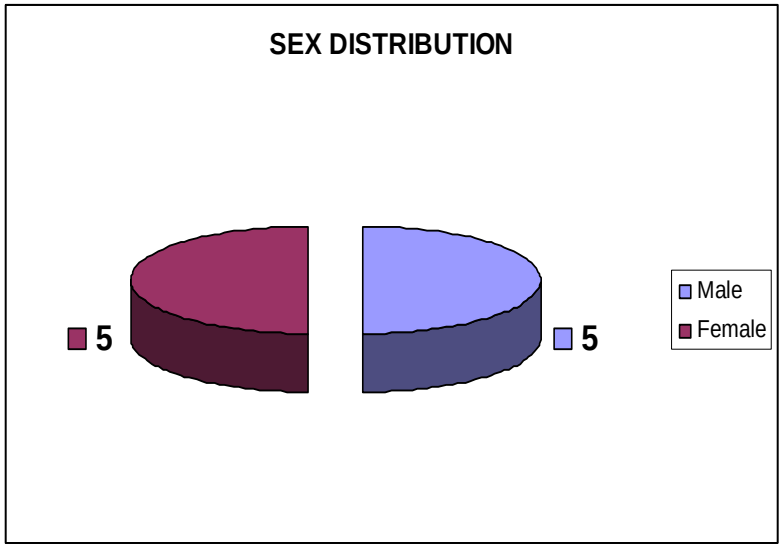
- To study the role of Chemotherapy in 4S neuroblastomas.

MATERIALS & METHODS

This is a combined prospective and retrospective study done at Department of Paediatric Surgey,ICH, spanning a four year period from 1st June 2001 to 30th May 2005.All case records of the 52 patients with neuroblastoma ,who presented within this period were taken up for he study.In this, we had ten cases of stage 4S Neuroblastoma .The youngest age of presentation was 2 months.The oldest patient was 11 months.



Sex distribution was equal,comprising gof five male and five female.The mode of presentation varied widely,commonest being an abdominal mass.



INCLUSION CRITERIA

1. Age < 1 yr
2. Histological evidence either from primary or secondary.
3. Imaging studies (Xray /USG / CT Scan) to prove presence of stage I ,II disease in Adrenals or sympathetic chain with secondary confined to liver and marrow (< 10 % involvement) and absence of disease elsewhere.

Urine 24 hr VMA positivity was a supportive evidence in all cases though it was not an absolute necessity to confirm stage 4S disease.

The investigation protocol was as follows. Tissue diagnosis was made based on wide bore needle aspiration of bone marrow in 7 cases,ultrasound guided Tru cut biopsy in two and open biopsy in one case.Chest Xray was taken in all cases with plan for CT chest if any doubtful lesion on skiagram.There however was no need of a CT Chest, in any of these cases. Ultrasonogram abdomen was done in all the cases to detect abdominal masses and for liver involvement.Ultrasongram of the lesion was done in cervical tumors. CT Abdomen was done in 4 cases during initial workup. Complete skeletal survey was done in all cases at presentation with bone scan prescribed for doubtful lesions.24 hour urine VMA was done in all cases.

In the observation group cases were followed up with bi weekly ultrasonograms of the lesion / abdomen and urine VMA studies. Bone Marrow was repeated once, the VMA became negative.Tumor regression and disappearance on ultrasonogram was confirmed by CT scan in all cases. Once tumor regressed the children were followed up with monthly ultrasonogram and 24 hour urine VMA.

The study group was divided into two.Group I ,which did not receive any chemotherapy and was kept on close followup.Group II which received chemotherapy. The indications for chemotherapy varied from random situations to specific indications.

Chemotherapy protocol we follow is a three drug regimen of intravenous cisplatin (100mg /m2), vincristine (1.5 mg/m2), cyclophosphamide (1000mg/m2) for each cycle over 3 days in divided doses.This cycle is repeated at 3 weekly intervals for a total of 6 cycles.

Case Studies :

The 10 cases have been divided into two groups.Group I, which did not receive chemotherapy (Cases I to V),Group II which received chemotherapy (Cases VI to X).

Case I :

2 months old female child presented with hepatomegaly, imaging revealed multiple nodules in the liver suggesting secondary deposits.No other mass detected in the abdomen.Urine VMA was positive, Bone marrow aspiration was positive for

clusters of round cells with rosette formation.Primary site was not detectable. Child was observed. Complete regression was documented at eight months of age.

Case II :

Two months old male child with massive hepatomegaly and in respiratory distress. Ultasonogram and CT showed multiple lesions in the liver,suggestive of Hemangioendothelioma. Laparotomy and hepatic artery ligation was done in view of the emergency situation for decompressing the liver .The diagnosis at this stage being hemangioendothelioma.But,biopsies taken form liver lesions showed neuroblastoma.

Urine VMA was positive.Bone marrow aspiration was positive for neuroblastoma.Child improved symptomatically and was kept on observation.Here also,the primary was not detectable.The disease regressed completely at 9 months of age.

Case III

8 months old female with a neck swelling,biopsy showing neuroblastoma. Liver ,bone marrow and VMA were positive.child was observed. Disease regressed completely at 1 year and 2 months of age.

Case IV :

7 months old male child with right adrenal mass on ultrasonogram.Bone marrow and VMA were positive.Child was kept on observation .Disease regressed at 12 months of age.

Case V :

4 months old female child with multiple skin nodules.Ultrasonogram showed a paravertebral mass.VMA and BMA were positive.During the observation period,new skin nodules appeared.hence chemotherapy was contemplated.However due to low platelet count ,chemotherapy was not given.Surprisingly,within a couple of weeks nodules started regressing and completely disappeared.Disease regression was documented at 13 months of age.

Case VI:

11 months old female with an abdominal mass, Ultrasonogram showed a left lumbar paravertebral mass.Bone marrow and VMA were positive. Chemotherapy given. BMA and VMA were negative by 1 month.Mass showed gradual regression and total regression of mass,occurred at 1 year and 6 months of age.

Case VII:

4 months old female child ,presented with hepatomegaly Ultrasonogram revealed a right lumbar paravertebral mass. Bone marrow and VMA were positive. Chemotherapy was given. Bone marrow and VMA reverted to normal in two weeks. Mass did not show regression in early phase. Infact,there was an increase in size of mass by 10 % ,in the first 4 weeks followed by slow regression. But by 10 months, the mass totally disappeared.

Case VIII :

Two months old male child, presented with an abdominal mass. .Ultrasonogram and CT revealed bilateral Adrenal masses ,each measuring 5 to 6 cms.Hepatomegaly present .BMA and VMA positive.Chemotherapy was given.BMA and VMA negative by 1 month.Adrenal masses came down by 1 cm in the first two months.Complete tumor regression was documented at 14 months of age.

Case IX :

8 months old male child ,presented with hepatomegaly and a right adrenal mass .VMA and BMA positive.New skin nodules started appearing. Chemotherapy was given.VMA became

negative by 1 month.BMA negative by two months.Mass showed no regression at 1 ½ years of age . With the mass persisting ,laparotomy and excision was done. Hisptopathology was Ganglioneuroma.

Case X :

3 months male child with massive hepatomegaly and respiratory discomfort . Right adrenal tumor was detected by ultrasonogram. BMA and VMA were positive.Chemotherapy was started.,Bone marrow and VMA were negative by six weeks. Mass showed slow regression.Complete regression was documented at 9.5 months of age.

GROUP I

Summary of Cases which were observed without any active treatment.

	Case I	Case II	Case III	Case IV	CaseV
Age	2mths	2mths	8 mths	7 mths	4 mths
Primary	Undetected	Undetected	Cevical	Adrenal	Lumbar
Secondary	Liver, BM	Liver, BM	Liver,BM	Liver,BM	Liver,BM,Skin
VMA	+ ve	+ve	+ve	+ve	+ve
Treatment Plan	Observation	Observaion	Observation	Observation	Obser
Regression	9 months	9 months	14 months	12 months	13 months
Maturation	--	--	--	--	--
Follow Up (age)	1 year	2 years	1 year	Lost	Lost

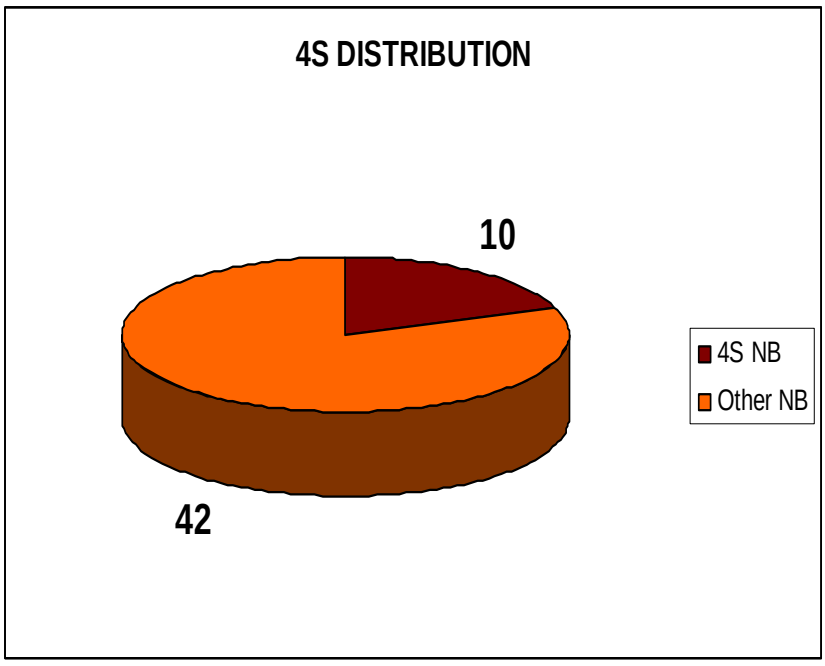
GROUP II

Summary of Cases which received Chemotherapy.

	Case VI	Case VII	Case VIII	Case IX	CaseX
Age	11mths	4mths	2mths	8 mths	3 mths
Primary	Lumbar	Lumbar	Bil.Adrenal	Adrenal	Adrenal
Secondary	BM	BM,Liver	BM,Liver	BM,Liver,Skin	Liver,BM
VMA	+ ve	+ve	+ve	+ve	+ve
Treatment Plan	Chemo	Chemo	Chemo	Chemo	Chemo
Regression	1 ½ yrs	10 months	14months	--	9.5 months
Maturation	--	--	--	At 1 ½ yrs	--
Follow Up (age)	2 ½ yrs	Lost	1 year	Lost	1 ½ yrs

RESULTS

Over the past 4 years we have had 52 cases of neuroblastomas.18 have been less than 1 year at presentation. Out of these 10 fit into the 4 SNB though some were not classified so at their first presentation. Six out of these cases presented less than 6 months of age. This forms a special subset as we will see in the discussion to follow. Male : Female ratio was 1:1



Group I :

There were few unique presentations as in Case II which presented with massive hepatomegaly with respiratory distress. Imaging studies showed multiple hypoechoeic well circumcised lesions suggestive of a hemangioendothelioma.in view of the worsening distress caused by massive liver,hepatic artery ligation was performed as an emergency procedure.the biopsies taken from liver lesions showed Rosettes confirming a Neuroblastoma.No primary was detectable.urine VMA +ve.The child was observed ,liver regressed lesions disappeared and VMA also became negative.

In case V, skin nodules were the presenting complaint and turned out to be a 4SNB.During followup new skin nodules appeared.Just when CT was contemplated ,the nodules started regressing and CT was deferred.Complete tumor regression ws documented at 9 months of age.

Case I, Case III and Case IV were classical 4S presentation with complete tumor regression.

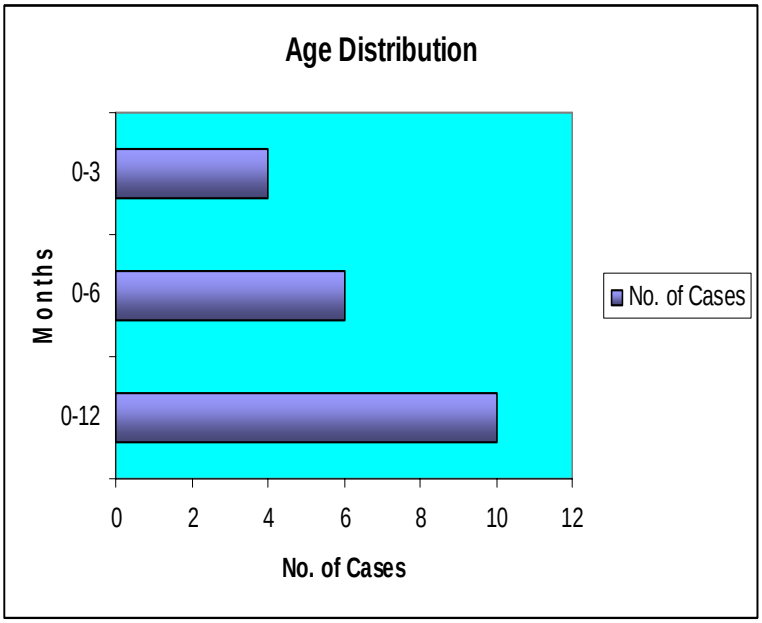
Group II (Chemotherapy group)

These patients were chosen for chemotherapy,both randomly and based on a few indications.In case VI, the age was > 10 months at presentation.This was considered as borderline age group.The benefit of doubt given to patients and chemo given.

Case VIII had Bilateral Adrenal tumor and was considered as poor risk group. Liver showed nodules on USG.BMA was +and VMA was also +.This was considered as Stage 4 disease at the outset and chemotherapy started. Extensive search of literature did not provide information about which group they could be included and how to stage them.The Bilateral tumors as like all 4SNeuroblastomas started showing regression and completely regressed at 1 yr of age.So we have reclassified them as 4S Neuroblastomas.

DISCUSSION

Of the total of 52 cases ,10 are 4 SNeuroblastomas.which constitutes 18.%. Of the 10 cases, 6 presented under 6 months of age. This forms favourable prognostic group within 4 S Neuroblastomas.Studies have shown 1 to 2 %of 4 SNeuroblastomas progress to stage 4 disease.UKCC group 1999 study shows this disease progression rate to be < 0.5 % in the under 6 months group.It is 3 to 4% in the over 6 months group.This is of great statistical significance,as it implies a two to three fold increase in disease progression rate .



Next is the under 3 months age group. Four out of the 10 presented at or less than 3 months of age and all of them did very well with complete disease regression,documented within 1 year of age in all except one. Review of literature shows this is a bad prognostic group as they tolerate the effects of diease poorly.particular problems being hepatomegaly causing respiratory compromise and coagulopathy.

Other complications caused by the enlarging live are renal failure, bowel ischemia,vomiting and aspiraton ,all caused by pressure effects over surrounding organs.In our series all 4 had massive hepatomegaly with respiratory distress in two of them.One of the cases,liver had HA ligation,the initial diagnosis being hemangioendothelioma.However all of them progressed well with good supportive treatment.

Case 8 is a very special one as it presented with bilateral adrenal masses with the disease spread pattern fitting into 4 S criteria.extensive search of literature did not reveal information about classifying such tumors.So,they were treated as stage 4 disease with chemotherapy.However they showed remarkable tumor and disease regression similar to that of 4S neuroblastoma.

Moreover the disease pattern suggested that chemotherapy had no great role in the disease regression.Thus the bilaterality of disease does not seem to affect staging as far as 4 SNB is concerned.

There are two concepts in 4 S Neuroblastomas as far as disease pattern is concerned and activity is concerned—tumor regression,tumor maturation.Both signify regression of disease activity and form the basis of the natural history of stage 4 S neuroblastomas.

Tumor Regression :

Tumor regression is defined as complete cessation of disease activity ie both primary,secondaries and their effects.This includes complete disappearance of the primary mass as well as manifest secondary disease,Next is reverting back of histology to normal.VMA also becomes negative.The disappearnce of mass lesions either the pimary or Liver lesions has to be documented by CT scan.Similarly histological and Biochemical markers negativity have to be proven by appropriate biopsies and 24 hour urine VMA tests.Various studies done during regressing phase in such tumors has shown APOPTOSIS to be the central event in tumor regression.

“Apoptosis” is described as programmed cell death.It is otherwise known as “cell suicide”, since it apparently involves active metablim by the dying cell. The sequence of leading to apoptosis is still unclear.But,it is currently thought that the process requires activation of an endogeneous endonucleus, perhaps via increases in cytosolic calcium, which results in fragmentation of DNA and subsequent cellular changes.

Though apoptosis is a physiological event ,it can also be triggered by pathological stimuli,such as irradiation and viruses. It is based on this knowledge that studies are on to investigate triggering factors of apoptosis which,in turn will lead on to disease regression.

Apoptosis is characterized histologically by chromatin condensation,cytoplasmic blebbing and electrophoretically by DNA fragmentation.Of these,the DNA fragmentation is considered most representative and also easy to observe.On the electrophoretic chart,this is shown by multiple horizontal bands at various levels, looking like a ladder. This ladder pattern of DNA fragmentation,is taken as gold standard for apoptosis.

Another means of establishing apoptosis is by analyzing the proliferative activity of tumor cells.This can be done by immunostaining of specific protein marker—PCNA (Proliferative cell Nuclear antigen)

Anitbodies directed against PCNA have shown a marked decrease in the number of actively proliferating cells among the tumor cells.

Histological proof from regressing tumors show tumor cells in various stages of apoptosis . However the factors which induce these changes have not been identified.And for the same reason the predictability of disease progression or regression becomes a problem.No definitive events or factors have been proven to predict the disease pattern in these patients.

Though apoptosis was documented in two of our cases,we have depended predominantly upon imaging and routine HPE negativity for establishing tumor and disease regression.

Tumor Maturation

Tumor maturation is defined as progression of the tumor cells from less differentiated to better differentiated forms.In other words,from a malignant histology to a benign histology.Thus the histological forms identified are from intermediate ganglioneuroblastoma to a benign ganglioneurom

In one of our cases despite chemotherapy the mass persisted for > 8 months in almost same size. On Laparotomy and excision biopsy we found the histopathology to be ganglioneuroma. This tumor maturation has been attributed to many specific factors which are expressed by the Neuroblastoma cells. The most important of them being NDGF (Nerve Derived Growth Factor).

The presence of these along with their receptors has been proven to induce differentiation amongst the neuroblastoma cells. The expression of these factors depends upon the presence of Trk gene. This gene is one of the favourable progrostic factors in NB. Thus it correlates well with this concept of tumor maturation. Thus it also serves as a predictor of disease regression in these patients.

In our study,we have had nine cases with documented disease regression and one with tumor maturation.The duration taken for disease regression form the time of presentation has varied from 5 months to 10 months. Overall the average duration for disease regression was 6.8 months.(27 weeks). In group I ,it was 6.5 months and in group II,it was 7.5 months.Thus the duration taken for disease regression in the chemotherapy group was slightly longer than in the no chemo group.

Out of all these cases,in six cases the pattern of disease has been stringently observed.Based on these observations,three phases of disease regression have been identified.

Phase I : Regression of Secondary disease .

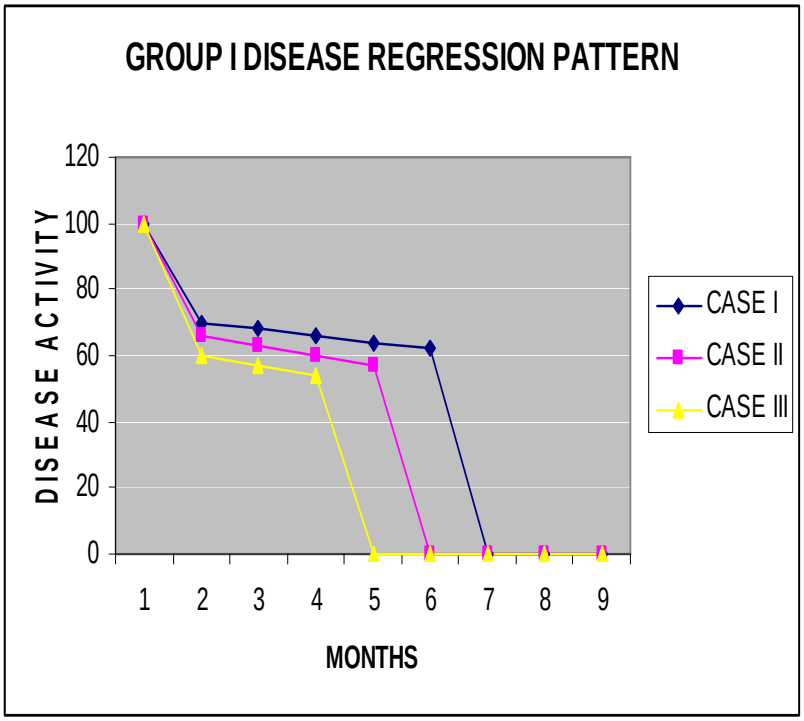
This was the earliest phase during which the skin nodules and liver metastasis disappeared.The bone marrow which was initially positive became negative.Urine VMA also became negative.

Phase II : Slow phase of Mass regression.

During this phase there was slow regression of primary mass by 10 to 20 % .

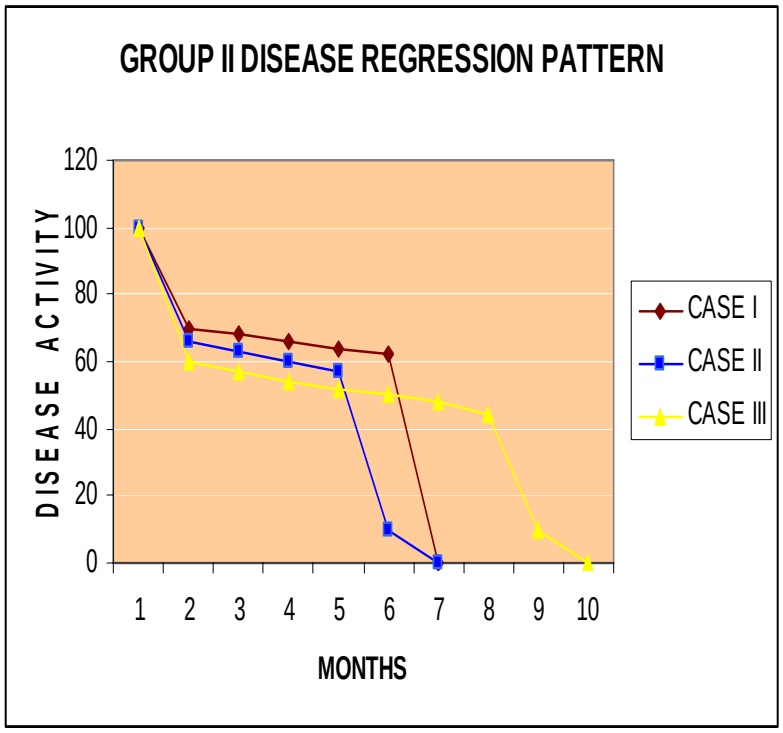
Phase III : Rapid phase of Mass regression:

During this phase the mass regressed rapidly and disappeared.



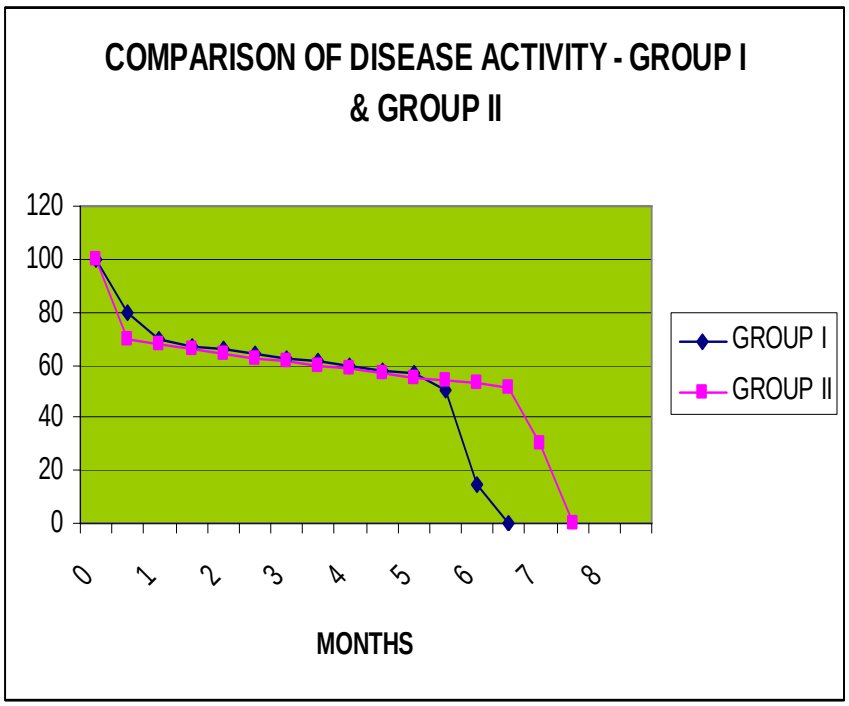
On an average the phase I in these six cases ,lasted about 10 % (2 to 3 weeks) of the total duration of disease regression. (27 weeks). There ,however was significant difference in phase I duration amongst the two groups.In the Group I patients, Phase I lasted for 3 to 4 weeks. Where as, in the Group II,it lasted for only two weeks before going into phase II.Thus chemo can be claimed to have shortened the phase I duration,though it did not

have any bearing in the total duration of disease regression.



The phase II duration was 12.5 weeks in Group I and 18 weeks in Group II.This being the longest phase of the three, reflects the increased duration,taken for disease regression in Group II patients.

The duration of Phase III did not vary much among the two groups – 3 weeks in Group I and 2.5 weeks in Group II.



Another point for discussion is disease progression or appearance of new lesions.In our study we have had one case where the mass has been progressively enlarging after presentation .Review of literature shows any such changes in volume of disease does not have a great bearing in the under 6 month age group.This is because any disease presenting less than 6 months of age is expected to reach a nidus before entering the regression phase.It is for this reason this group falls into good prognosis group.

Next comes the major decision of whether to actively manage these patients or not. The options are surgery and

chemotherapy.Various papers have been published on antenatal detection of NB's.All these have led to detection of more and more masses and their early intervention before they enter into their natural regression pattern.

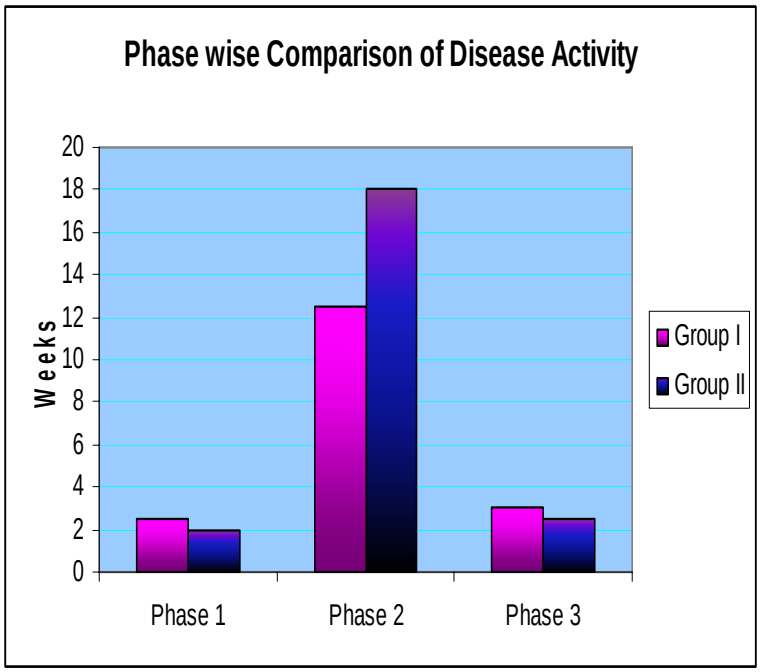
These studies with other comparative studies have shown, such early detection and intervention was over treatment.This is because,they,when allowed in their natural course were found to regress spontaneously.There however is a 1 to 2 % chance of these cases going into stage 4.This has to be weighed against the morbidity and complications of surgery and chemotherapy.

Studies published so far have proven beyond doubt that surgery does not have any role in prevention of disease progression.Thus surgery does not have any role in the early stages of 4SNB atleast.

The next option is chemotherapy.There have been varying reports for and against chemotherapy in these case.In our study,we have compared two groups,the chemotherapy group Vs the no chemotherapy group.

The indications for chemotherapy in our group were random selection in 3 cases ,borderline age group in one case and bilateral tumor in another case.

The chemotherapy protocol was half dose PVC regimen,at 3 weekly intervals for 6 weeks. The observations were ,the average period for phase I was two weeks, the average phase II duration was 24 weeks and Phase III duration was 3 to 4 weeks. One of the chemotherapy group went in for tumor maturation.



Overall,there were no significant changes observed in the pattern of disease regression between the two groups, excepting

the phase I duration. Thus in our study chemotherapy ,did not seem to induce tumor regression or maturation.Neither did it reduce the duration of disease . Thus,the role of chemotherapy is limited to specific clinical situations.

In our setup where N-mycamplification and DNS studies are practically not possible,we have defined a clinical protocol.Any 4S NB presenting after 6 months of age with evidence of progression of disease or non regression of disease might need chemotherapy. Progression of disease is defined as increase in size of primary or appearance of new lesions. Non regression of disease is defined as failure of progression from phase I to phase II in a time frame of two months.

CONCLUSION

This study of 4S Neuroblastomas in our institution over past 4 years establishes the following things. In our study all cases (100%) have shown either tumor maturation or tumor regression. The subgroup of $\leq$ 3 months have done remarkably well in our study unlike other studies. The secondary effects which are the causes of morbidity and mortality in these cases have not been observed in our study group.

Most importantly, a set disease pattern has been identified in the 4 S Neuroblastomas. Though the duration of disease regression varied with an average period of 6.8 months.All these cases followed a set pattern with three phases. Phase I of regression of secondary disease. Phase II of slow tumor regression and phase III of rapid tumor regression.

With regard to the role of chemotherapy in these cases, our study concludes there is no role for chemotherapy in these cases as a routine. Chemotherapy does not seem to alter the duration of disease neither does it initiate the process of maturation or tumor

regression. In fact the duration of disease regression has been slightly higher in the chemotherapy group.

Based on all these we formulate the following indications for chemotherapy in 4SNB.

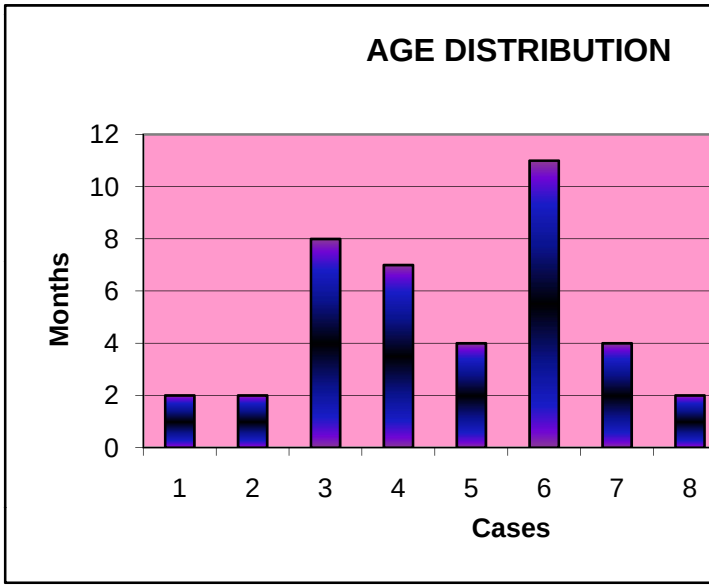
Age > 6 months as presentation with

1) Disease progression in form of new lesions / enlarging masses.
2) Failure of disease regression identified by the non progression from Phase I to Phase II.

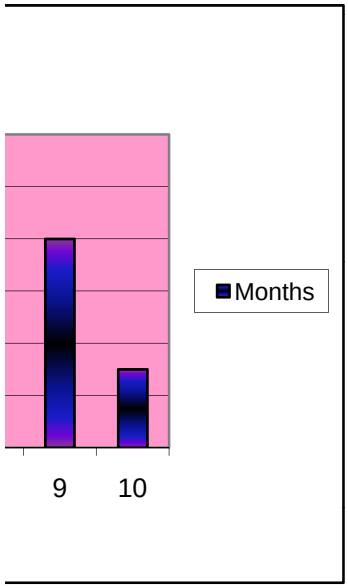
This six month age cut off has been proposed based on studies proving increased chances of disease progression,in cases presenting beyond this age.

Apart from these, any secondary effects of disease,which is life threatening (Eg, rapidly enlarging liver),warrants chemotherapy,in any age group.

Cases	1	2	3	4	5	6	7
Months	2	2	8	7	4	11	4



8	9	10
2	8	3



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